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54. Name of Invention: Cellular Classification Device

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Detailed Description

Name of Invention: Cellular Classification Device

Patent Application Details

1. A cellular classification device having the characteristics of a first investigation method in which light is shown on a cellular sample comprised of at least one cell, and light information emitting from the cell is captured and read by a low-powered microscope, and a first discrepancy method in which the information gathered in the said first investigation method showing the specific characteristics of the cell is compared to the standard values for the said cell, and those values are classified based on the range of standard values in which they fall, and a memorization method in which the position of the cells within the cellular sample examined by the said discrepancy method which do not fall within the range of standard values is recorded and memorized, and a second investigation method in which light is shown on the said cells whose position has been recorded and memorized by the said memorization method, and the light information emitting from the cell is captured and read by a high-powered microscope, and a second discrepancy method in which the information gathered in the said second investigation method showing the specific characteristics of the cell is compared to the standard values for the said cell, and those values are classified based on the range of standard values in which they fall, and a display method in which the discrepancy classification results from the first and second discrepancy methods are displayed.

2. A cellular classification device outlined in Item 1 of the Patent Application Details for the invention having the characteristics of the light shown on the said cell

sample in both the first and second investigation methods is a visible light, and the light information thus acquired is image information emitted from the cell.

3. A cellular classification device outlined in Item 1 of the Patent Application Details for the invention having the characteristics of the light shown on the cell sample in the said first investigation method is a laser light, and the light information is fluorescent light information emitted from the said cell that has absorbed the said laser light beam.

4. A cellular classification device outlined in any of the Items 1 through 3 above of the Patent Application Details of the invention having the characteristic that the said cells are blood cells.

Detailed Description of the Invention

{Usage Applications for the Invention}

The current invention is a cellular classification device, and is particularly concerned with determining cellular images and classifying them into either normal or abnormal cells.

{Background of the Invention}

The original cellular classification devices were those in which, particularly for the classification of blood cells, a stained blood sample was set onto a 600 to 1,000 magnification high-powered microscope, the structural images of the blood cells scattered in the sample were analyzed one by one using a TV camera, then the classification of the blood cells was performed using a distinctive classification method. However, when using a high-powered microscope for the discernment of blood cells, the number of cells that can be viewed in the field of vision of the microscope at one time is limited, and the amount of time it takes to distinguish and classify the cells taking up the squared area of the microscope field of vision is proportionately larger. Therefore, for a blood sample of about 300μm, with blood cells scattered proportionately in all four directions, in order to seek out particularly white blood cells, it takes about 0.5 ~ 1.0 minutes to classify and sort out about 100 white blood cells. In order to produce the statistical accuracy needed for the experimental results, 300 cells per sample must be examined and classified, and since there is a limit to the processing capacity of the classification device, even more time is required for this processing. Thus in an effort to improve the statistical accuracy of the abovementioned classification device, the processing time must necessarily be increased, and thereby processing capacity decreased. In order to resolve this problem, a new method of classifying white blood cells was developed in which, using the enzyme at the nucleus of white blood cells and a controlled enzyme that is different from the first but reacts with it, a laser light is shone onto the blood sample in normal liquid form, and the white blood cells thus illuminated are distinguished by the intensity of the light thereby refracted. This type of enzyme chemical response process (otherwise known as a flow method) was developed, and put into working form as a blood cell classification device (for an example, please see patent number S59-853).

However, whereas with the first mentioned blood cell classification device that classifies blood cells structurally, the cells are classified based on their structural characteristics and therefore correct information is obtained, a blood cell classification device based on the flow method classifies blood cells by measuring the intensity of light

and therefore can be used in place of the structural classification method for normal blood cells, but cannot be used for abnormal or immature white blood cells. Therefore, the problem remains that this method cannot be used for clinical testing currently being performed.

{Goal of Invention}

The goal of this invention lies in supplying a cellular classification device which can classify the necessary number of cells within a short measurement period with the required amount of testing precision and accuracy, and which has improved processing capacity.

{Outline of Invention}

The inventors had various discussions about the imagery analysis of cells, and came to the conclusion that if a low-magnification microscope is used for the distinction and classification of cells, the number of cells that fall within the field of vision at one time increase, and the time required to go from viewing one cell to the next is proportionately decreased by the square of the magnification factor. Since the amount of light information that can be discerned when using a low-magnification microscope is lower, the special characteristics of the cells cannot be extracted, and classification cannot be performed. However, if a high-magnification microscope is used, the amount of light information is increased, but the measurement time required also increases. The inventors focused on this point, and arrived at the conclusion that if they first use a low-magnification microscope to perform the analysis and rough classification accurate to the extent possible with the cells, they can then use a high-magnification microscope to perform precision classification of the cells that could not be classified correctly through the low-magnification microscope. This solution would thus satisfy the stated goals of the invention. Namely, this invention consists of a cellular classification device having the characteristics of a first investigation method in which light is shown on a cellular sample comprised of at least one cell, and light information emitting from the cell is captured and read by a low-powered microscope, and a first discrepancy method in which the information gathered in the said first investigation method showing the specific characteristics of the cell is compared to the standard values for the said cell, and those values are classified based on the range of standard values in which they fall, and a memorization method in which the position of the cells within the cellular sample examined by the said discrepancy method which do not fall within the range of standard values is recorded and memorized, and a second investigation method in which light is shown on the said cells whose position has been recorded and memorized by the said memorization method, and the light information emitting from the cell is captured and read by a high-powered microscope, and a second discrepancy method in which the information gathered in the said second investigation method showing the specific characteristics of the cell is compared to the standard values for the said cell, and those values are classified based on the range of standard values in which they fall, and a display method in which the discrepancy classification results from the first and second discrepancy methods are displayed. The classification device also has the characteristic that it is able to perform the distinction of the number of cells required for statistical precision and accuracy in a very short amount of time. This invention uses visible light

rays, ultraviolet rays, infrared rays and laser light rays for the light to illuminate the cellular sample. The selection of which type of light source to use can be based on the special characteristics of the cells that are to be classified and measured. Based on the light source chosen, the type of microscope and inspection gear used to read the light information from that microscope is called out. For example, when using a laser light, a fluorescent microscope is used for the microscope and fluorescent inspection gear is used for the inspection gear. For visible light, a normal microscope is used for the microscope and a TV camera for the inspection gear.

The fundamental principles that made the above invention possible will now be explained using a cellular classification device that classifies blood cells as an example.

There are five types of white blood cells distributed in a blood sample; normal white blood cells are neutrophils, lymphocytes, monocytes, eosinphils, and basophils. In addition, there are stem cells (芽球), myelocytes, and red stem cells (赤芽球), which are immature blood cells or abnormal lymphocytes that appear during sickness. In a normal blood sample, the white blood cells are diffused over a 200 ~ 300μm area in four directions. About 90% of the cells are neutrophils and lymphocytes, with monocytes, eosinphils and basophils present in much lesser quantities. Also, white blood cells stained with light dye or [meigimza] dye through normal dyeing methods display their structural characteristics such as the color of cytoplasm or granulocytes, shape of the nucleus and size, based on their cell type. The average diameter of a white blood cell from a sample is around 10μm ~ 20μm. Originally, an optical microscope with a 40 ~ 100 magnification clinical lens was used to split the white blood cell images into 0.25μm ~ 0.5μm four-directional minute pixels. The concentration of color of these pixels was then analyzed and characteristics of the blood cell discerned. Through this method, white blood cells were correctly distinguished and classified.

Using the typical characteristics of white blood cells such as the color of the cytoplasm, the color of granulocytes, the shape of the nucleus, and the size of the nucleus and cytoplasm, typical white blood cells are first taken up, and setting even normal cells which have been deformed as unknown cells, it is possible to perform a correct preliminary rough distinction of at least the typical neutrophils and lymphocytes, which encompass a major portion of the white blood cells. This process of preliminary rough distinction is sufficient for cell subdivision capacity of less than 1μm and so therefore should be feasible using a high-speed image processing device consisting of a low powered microscope with a 20-magnification object lens, and a high reduction capacity linear array semiconductor inspection device with a breakdown capacity to less than 1μm, to expect a blood cell processing speed of about 10 ~ 20 times faster than the original. Next, the positions of the blood cells marked as unknown using the preliminary rough classification in the sample are memorized, the sample is moved to a high breakdown capacity microscope as in the original method, and the marked cells are analyzed and processed with precision. The blood cells that require such precision classification processing are very few, thought to be only about one-fifth to one-tenth of the total number of white blood cells in the sample.

In addition to the above, white blood cells can also be classified at high speed using a sample double-stained with fluorescent dye and regular dye. Fluorescent dye, for example, has a different dye hue depending on the type of white blood cell, or use a unique dye for each type of white blood cell such as a dyeing method where a blue

colored laser shone on a white blood cell brings out a particular fluorescent light, the wavelength and intensity of the light emitted from the white blood cells is analyzed, and through this the white blood cell type and information about sickness can be discerned. With fluorescent dye, the nucleus material of the white blood cells is stained, and based on the cell type, there could be monoclonal antibodies displaying immunization phenomena such as [akurijin] orange fluorescent test fluid, T-lymphocytes, B-lymphocytes, and immature cells. An important consideration for this double dyeing method is that after dyeing the blood with fluorescent dye, it was then stained with normal dye, thus creating a double-stained sample. First, a low powered fluorescent microscope is used to analyze the fluorescent light emitting from the white blood cells in the blood sample, and the white blood cells are classified according to their fluorescent analyses. Unique blood cells such as immature cells, T-lymphocytes and B-lymphocytes are similarly determined. At this fluorescent analysis stage, the five types of normal white blood cells can be roughly determined and classified, abnormal cells and cells which require special inspection can be determined and the position of these duly recorded and marked. Next, a high-powered microscope is used to structurally distinguish and classify those blood cells that could not be characterized with the fluorescent microscope and the cells requiring further inspection. In this way, through a combination of a fluorescent analysis method and a structural distinction method, it is possible to perform distinction and classification with high accuracy and confidence using the compound information from the cells' identities that could not be had from original classification methods. There is also the characteristic that new diagnostic information about an illness can be obtained by contrasting it with structural information.

Unlike as with the previous structural classification method, there is no need to determine the structure and shape of the blood cells when using a fluorescent microscope for blood cell classification and therefore it is possible to reduce the magnification of the microscope, and one can expect a significant improvement in the inspection processing capability over the original method.

{Example of Functional Application for the Invention}

Next an explanation will be given of a desirable functional application for this invention using the appended figures.

Figure 3 shows white blood cells on a blood sample being image processed by a low magnification microscope or a low powered fluorescent microscope, or a microscope used to perform rough distinction and classification through a fluorescent analysis method in a combined field of vision, and a precision distinction and classification by a high magnification microscope in a combined field of vision.

In Figure 3, 31 is the blood sample, 32 is the white blood cells scattered in the sample and their location positions xiyi, and 33 represents the field of vision of the low magnification microscope. 34 shows the field of vision of the high magnification microscope, and 35 represents the field of vision of the low magnification fluorescent microscope. The low magnification field of vision in 33, for example, if the object lens of the microscope were set to a magnification of x20 would be about 1,000μm square, and would include an average of 8 ~ 10 white blood cells scattered within it. In order to perform the classification of these white blood cells through image processing, the smallest possible capturable image unit or pixel is required, namely about $1\mu m^2$, as in 36.

There is a process for scanning the field of vision screen using an inspection device for image capture such as a 1024 linear array semiconductor detector. As another example, there is a method where, using a 1024 x 1024 area array semiconductor camera and moving the field of vision intermittently, images of the white blood cells can be captured at a higher rate of speed while simultaneously recording their positions. In the case of a high magnification field of vision as shown in 34, a pixel of from $0.25\mu m^2$ to $0.5\mu m^2$ is required, and a microscope with a x40 ~ x100 magnification object lens can be used to capture the images as in the original method. With the low magnification fluorescent analysis field of vision in 35, the white blood cells are taken as the light source of the fluorescent light so it is sufficient to know the location position, and therefore a low magnification microscope can again be used. For example, if a microscope with a x10 magnification object lens were to be used, the diameter of the field of vision would be 2,000μm and about 30 ~ 40 white blood cells would be scattered within the limits of the field. In this case, the white blood cell fluorescent inspection device differs from the image capture in 33, in that there is a need to get the highest possible fluorescent inspection sensitivity of the white blood cells, so instead of making the size of the pixel in 38 smaller than the white blood cell size, it is desirable to make it about the same size as the cell itself. Therefore, it is possible to use a low-priced 512-channel to 256-channel low reduction capacity semiconductor inspection device as a linear array inspection device that is made for pixels slightly smaller than the nucleus of a white blood cell, $4\mu m^2 \sim 8\mu m^2$. To further increase the processing speed, a camera with a 512 x 512 or a 256 x 256 area array semiconductor inspection device can be used. When using a regular camera, it takes 16 milliseconds to scan the area of one field of vision, so if a fluorescent microscope with a x10 magnification object lens is used to classify white blood cells via the fluorescent analysis method, the 30 or so white blood cells falling within a single field of vision could be analyzed in just over 16 milliseconds. Adding in the time it takes to adjust the microscope stage, auto focus and receive and process information from the area, if it took twice the time, one field of vision could be analyzed in 32 milliseconds, allowing for the analysis of 900 white blood cells in one second. Therefore, it is thought that the processing speed of this classification system invention is affected by the classification speed of the high magnification microscope at the latter stage. If it is assumed that the number of white blood cells that need to be classified at the latter stage is equal to 10% of the total, then when there are 1,000 white blood cells processed by fluorescent analysis at the former stage, 100 of them would require further image analysis at the latter stage. Because the locations of white blood cells requiring latter stage imagery analysis are already marked at the former stage, the same amount of time to search out white blood cells scattered in the sample is not required as in the original method. The microscope stage can be controlled to move directly to the position of the cells but 0.1 seconds would still be required for this. Ten seconds would be required for processing 100 white blood cells, and even if the time to change samples is added in, over 200 slides could be processed in one hour. With the original method, processing 100 slides containing 100 white blood cells each in an hour would be considered high speed processing, so the new method is an improvement in processing capacity of about 20 times. However, the number of blood cells requiring imagery analysis determines the processing capacity.

Figure 4 shows the fluorescence emitted from white blood cells stained in [akurijin] orange as a fluorescent dye when illuminated with a 488 nm blue color A1 laser with a blue color, and the distribution frequency of the various types of white blood cells. In the figure, (a) shows a white blood cell spectrograph when illuminated by a fluorescent green color with a wavelength of about 530 nm, and (b) shows a white blood cell spectrograph when illuminated by a fluorescent red color with a wavelength of about 650 nm. Also, B are basophils, M are monocytes, E are eosinphils, N are neutrophils and IMM are immature cells. From the two-dimensional luminosity spectrograph of these two fluorescent colors, it is possible to classify the five types of typical white blood cells and immature cells.

Figure 1 shows a structural drawing of an actual application of the cellular classification device in this invention, where the blood sample is stained with normal dye, and both low magnification and high magnification microscopes and an image processing method is used for rapid processing in the blood cell classification system.

In the figure, various samples 2 are introduced from sample autoloader 1 to the ID reader 3 on the way to the stage of low magnification microscope 4. Light source 5 that illuminates low magnification microscope 4 with visible light and camera 6 are connected to low magnification microscope 4. Said camera 6 is connected to A/D transformer 7, and this A/D transformer 7 is connected to image memory 8. Said image memory 8 is connected to characteristic recording device 9, and characteristic recording device 9 is connected to blood cell differentiation computer 10. Said blood cell differentiation computer 10 is connected to ID classification result memory, and this memory is connected to printer 12.

X – Y controller 13 is connected to said low magnification microscope 4, and sample ID unknown blood cell location memory 14 is connected to this controller 13.

Said ID reader 3 and blood cell differentiation computer 10 are connected to sample ID unknown blood cell location memory 14, and this memory 14 is connected to ID checker 15. This checker 15 is connected to ID reader 16. X – Y controller 16 is connected to said ID checker 15, and high magnification microscope 17 with light source 19 is connected to this controller 16. Camera 18 is connected to high magnification microscope 17, and A/D transformer 20 is connected to this camera 18.

Said A/D transformer 20 is connected to image memory 21, and said characteristic recording device 9 is connected to this image memory 21.

Next the operation of this functional application will be explained. First, blood sample 2, which has been stained with [meigimza] dye, is moved by autoloader 1 past ID reader 3, where the ID of the sample is read and recorded, and is mounted onto the X – Y stage of low magnification microscope 4 with visible light illuminating from light source 3. This X – Y stage is then controlled by controller 13, and after auto focus, the location and image of white blood cells scattered in the each of the fields of vision one by one are recorded by camera 6 with the white blood cell inspection device on microscope 4. The image numbers recorded by said camera 6 are translated separately by the location of each blood cell into red (R), green (G) or blue (B) density signals fixed inside of camera 6, and because these signals are processed based on their digital quantities, the signals are digitized in A/D transformer 7. These digitized signals are then recorded in image memory 8. During the time the microscope 4 stage is moved to the next field of vision

and is preparing for the image capture, image memory 8 records the size, shape, and color intensity characteristics of the white blood cells using characteristic recording device 9.

Next the blood cell differentiaton computer 10 records the data gathered by characteristic recording device 9, the calculation of white blood cell area, the detection of the largest and smallest values, intensity histograms, the perimeter, the number of subdivision are all calculated and processed, and through a differentiation function, everything is compared to the recorded values and the classification of the various types of white blood cells is performed. Through this type of process, the number of blood cells required for the accuracy of classification inspection is differentiated and those results recorded by ID number in classification results memory 11. At the same time, blood cell differentiation computer 10 records the locations of any abnormal or unknown cells in blood cell location memory 14. Next, ID reader 16 and checker 15 carry sample 2 from autoloader 1 to high magnification microscope 18 illuminated by visible light from light source 19, and the sample ID is confirmed. Next those blood cells that were unknown or abnormal cells classified by the said low magnification microscope 4 and whose positions in the sample were recorded by location memory 14 are called up by X – Y stage controller 16, and the detailed information recorded by camera 18 through A/D transformer 20 and into the image memory 21. The movement of X – Y stage is done by a control device inside X – Y controller 16, but because the positions of unknown and abnormal cells were previously recorded in memory 14, the movement of the stage is very rapid. As X – Y stage controller 16 is calling up the next unknown or abnormal cell and preparing to record the image, the image memory of white blood cells are precisely differentiated and classified by characteristic recording device 9 through blood cell differentiation computer 10 on a shared time basis, and the results are sent to result memory 15.

Further, the operation of camera 18, A/D transformer 20 and image memory 21 are all the same as camera 6, A/D transformer 7 and image memory 8 as used with low magnification microscope 21.

In this way, after all unknown and abnormal cells are called up by X – Y controller 16, and differentiated and classified by high magnification microscope 17, the results are reported as final results for the sample by printer 16.

As in the above functional application example, by using the rough and precision differentiation and classification in two stages of blood cells on multiple slides, even if more than 300 white blood cells per slide are processed, a processing capacity surpassing the original method by two to many times can be expected. Therefore, hospital clinical inspection cellular classification of multiple blood stained slides can be performed at a high speed with good accuracy. This is particularly effective for urgent inspections.

In the functional application example, slide 2 was moved to high magnification microscope 17 by sample autoloader 1, but if necessary high magnification microscope 17 can also be moved to the sample portion.

Figure 2 is a structural drawing showing a differing layout from that in Figure 1 of a functional application example of the cellular classification device of this invention.

The difference between the functional application example in Figure 1 and this one is that in this example, sample 2 is double-stained with fluorescent dye and normal dye, the cellular sample illuminated by laser light, and rough classification performed on white blood cells then viewed through a log magnification microscope.

The structure of this functional application example and that outlined in Figure 1 differ in the following areas.

The light source 5 of visible light in the Figure 1 functional application example is replaced in this one by light source 22 of laser light, and this light source 22 is connected to low magnification fluorescent microscope 23. In place of camera 6 from the Figure 1 functional application example, fluorescent inspection device 24 is attached to said microscope 23, and A/D transformer 7 is attached to inspection device 24. In place of image memory 8 from Figure 1 functional application example, fluorescence intensity analysis device 25 is connected to said A/D transformer 7. This inspection device 25 is not connected to characteristic recording device 9 as in Figure 1 functional application example, but is rather connected directly to the blood cell differentiation computer.

Next the differences in operation between this functional application example and that in Figure 1 will be explained. The laser light from light source 22 brings out the fluorescent pigment from inside the white blood cells, and the fluorescence is ready by fluorescent recording device 24.

The fluorescent signals read by inspection device 24 are analyzed by their various fluorescent luminosities by green, red, etc. fluorescent filters located inside inspection device 24, digitized by A/D transformer, and changed to digital values. The various types of fluorescent luminosity signals that were changed into digital values are analyzed for intensity for each white blood cell location by fluorescent analysis device 25. Based on the spectrograph of each fluorescent luminosity wavelength, the type of each white blood cell is classified by blood cell differentiation computer 26 and those results recorded by memory 11. The positions of cells judged unclassifiable by blood cell differentiation computer 26 and immature cells are recorded by memory 14, and as shown in the Figure 1 functional application example, the white blood cells are then precisely imagery analyzed by high magnification microscope 17.

As shown above, the classification of blood cells using a combination of fluorescent analysis and image processing can be accomplished not only with two-stage microscope analysis, but also by using one microscope and simultaneously performing fluorescent analysis and image processing. In this case, rather than the increased processing speed for blood cell classification, the compound blood cell information allows for an increased amount of clinical diagnosis information and thereby an increased accuracy of diagnosis.

With the functional application example above, because laser light is used for the classification of blood cells, in the rough classification stage, it is possible use a lower powered microscope than that used in the Figure 1 functional application example. Therefore, it is possible to hold a higher accuracy in classification than in the Figure 1 functional application example, and yet reduce classification processing time.

{Results of Invention}

As explained above, with this invention, it is possible to rapidly classify the number of cells required for accurate cellular classification inspection results with this invention.

{Simple Explanation of Drawings}

Figures 1 and 2 are structural drawings showing functional application examples of the cellular classification device of this invention. Figure 3 are drawings showing the position of blood cells as part of blood samples and the field of vision of microscopes. Figure 4 is a graph showing the relationships between white blood cell frequencies in blood and fluorescent luminosities.

4 – low magnification microscope
6 – camera
8, 21 – image memory
9 – characteristic recording device
10 – blood cell differentiation computer
11 – ID and classification results memory
14 – sample ID and unknown cell position memory
17 – high magnification microscope
23 – low magnification fluorescent microscope
24 – fluorescent recording device
25 – fluorescent luminosity analysis device

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Figure 1

Figure 2

Figure 3

Figure 4

(a)
White Blood Cell Frequencies
Green Fluorescent Luminosity

(b)
White Blood Cell Frequencies
Red Fluorescent Luminosity